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Carbon-based nanocomposites with aptamer-templated silver nanoclusters for the highly sensitive and selective detection of platelet-derived growth factor

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ABSTRACT

We synthesized two kinds of carbon-based nanocomposites of silver nanoclusters (AgNCs). An aptamer for targeted platelet-derived growth factor-BB (PDGF-BB) detection was used as the organic phase to produce AgNCs@Apt, three dimensional reduced graphene oxide@AgNCs@Aptamer (3D-rGO@AgNCs@Apt), and graphene quantum dots@AgNCs@Aptamer (GQD@AgNCs@Apt) nanocomposites. The formation mechanism of the developed nanocomposites was described by detailed characterizations of their chemical and crystal structures. Subsequently, the as-synthesized nanoclusters containing aptamer strands were applied as the sensitive layers to fabricate a novel electrochemical aptasensor for the detection of PDGF-BB, which may be directly used to determine the target protein. Electrochemical impedance spectra showed that the developed 3D-rGO@AgNCs@Apt-based biosensor exhibited the highest sensitivity for PDGF-BB detection among three kinds of fabricated aptasensors, with an extremely low detection limit of 0.82 pg mL^{-1}. In addition, the 3D-rGO@AgNCs@Apt-based biosensor showed high selectivity, stability, and applicability for the detection of PDGF-BB. This finding indicated that the AgNC-based nanocomposites prepared by a one-step method could be used as an electrochemical biosensor for various detection procedures in the biomedical field.

1. Introduction

As a widely used biomarker for hepatic fibrosis, liver cancer, and gastrointestinal stromal tumors, platelet-derived growth factor (PDGF) has been implicated in the pathogenesis of angiogenesis in these tumor types (Wang et al., 2007). PDGF is a growth factor protein found in human platelets and has three isoforms: PDGF-AB, PDGF-AA, and PDGF-BB (Babu et al., 2013). Among them, PDGF-BB, an important cytokinein serum, is a protein marker for cancer diagnosis and directly involved in many cell transformation processes, such as tumor growth and progression (Hannink and Donoghue, 1989). PDGF-BB is close to some appalling diseases, such as atherosclerosis, fibrosis and often over-expressed inhuman malignant tumors serving as an indicator for tumor angiogenesis (Betsholtz et al., 1984). Therefore, cognition and quantification of PDGF-BB are particularly significant in biomedical fields. With the increasing demands of proteomic strategies for the clinical diagnosis and therapeutic analysis, the development of accurate, highly selective, sensitive, and facile detection of cancer-related proteins receives significant attention (Bezuidenhout et al., 2007; Liang et al., 2013; Wang et al., 2015). To date, various commonly available methods, such as enzyme-linked immunosorbent assay (Gersuk et al., 1989), fluorescence immunoassay (Huang et al., 2005), and chemiluminescence immunoassay (Huang et al., 2008), were developed for PDGF-BB detection. However, these methods are expensive, time consuming, and labor-intensive (Liu et al., 2015b). In the past few decades, electrochemical techniques have been receiving considerable interest for the detection of small biomolecules owing to their high sensitivity, rapid response, and low expense. For example, Fu's groups explored an enzyme-mediated direct electrochemistry for sensitive detection of PDGF. This proposed aptasensor shows a relatively low detection limit of 1.7 pM (Deng et al., 2013).

Aptamers are single-stranded DNA or RNA selected via an in vitro evolution process of systemic evolution of ligands through exponential enrichment (Yu et al., 2012). Aptamers have many advantages, such as a high level of both specificity and affinity, easy synthesis and storage, and flexibility in labeling at a desired site without loss of activity. Thus,

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aptamers are more suitable for protein detection than antibodies (Huang et al., 2008). Recently, combining the distinct properties of aptamers with various transducers, multiple sensing strategies, such as fluorescence (Lan et al., 2010), surface-enhanced Raman spectroscopy (Wang et al., 2010), microgravimetry (Freeman et al., 2009), quartz crystal microbalance (QCM) (Yao et al., 2009), electrochemiluminescence (Zhuo et al., 2015), surface plasmon resonance (SPR) (Chen et al., 2015), and electrochemistry (Li et al., 2015), have been developed. Among these, electrochemical aptasensors based on the specificity of aptamer-target recognition have received particular attention because of their high sensitivity and selectivity, simplicity, and economy (Liu et al., 2015b). Various nanomaterials have been applied for signal amplification, including gold nanoparticles (Gong et al., 2015), carbon nanostructures (Zhong et al., 2015), and quantum dots (Lim et al., 2015), to achieve highly sensitive electrochemical aptasensors. Nevertheless, nanomaterial application has many disadvantages, such as complicated procedures, high expenses, and poor reproducibility and quantification, particularly for the complex samples (Borm et al., 2006). Aptamer immobilization is one of the key steps that can be addressed to improve the biosensor response, thermostability, and long-term stability. Covalent immobilization of the aptamer could improve the amount of bound protein. Therefore, simple aptasensors for ultrasensitive and convenient detection of proteins are still an urgent demand.

DNA sequences can be changed and modulated easily; thus, DNA has recently emerged as an attractive template to fabricate inorganic nanoparticles, which possess distinct physical and chemical properties in optics, magnetic, electronic, and catalysis. In particular, DNA-directed silver nanocluster (AgNC) syntheses have been thoroughly investigated, and the strongly emissive AgNCs@DNA show a wide range of potential applications, specifically in biosensing. These materials may have some advantages, such as ease of synthesis, desirable photophysical properties, and a smaller size than quantum dots. They also allow flexible modification with aptamers or other recognition elements when they are used in biosensing or bioimaging fields. To date, AgNCs are widely used in the aspects of light scattering (Anand et al., 2012), fluorescence (Zhang et al., 2014b), chemiluminescence (Deng and Ju, 2013), and electroluminescence (Li et al., 2014) for their excellent optical properties. Despite the excellent properties found in AgNCs, some of their other significant properties, such as the electrocatalytic activity, are considered with little attention. Moreover, AgNCs are rarely used in the field of electrochemistry.

Carbon-based materials are the current most widely used nanomaterials for the fabrication of electrodes because of their semiconductive behavior and high porosity; these materials are also suitable for protein detection (Sarma et al., 2009). To the best of our knowledge, no report is available regarding the one-step synthesis of the carbon-related AgNCs@Apt and its application on the electrochemical biosensor for sensitive protein detection. Compared with previous colorimetric and fluorescence reports, the current strategy presents two advantages. First, unlike other sensitive layer of electrochemical aptasensors, the binding of probe aptamer with the nanomaterials is combined with the inorganic nanoclusters. Second, the application of carbon-based nanomaterials in AgNCs@Apt could improve the electrochemical performances and lead to high sensitivity of the developed electrochemical sensing system (Scheme 1).

2. Experimental section

2.1. Materials

Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) was obtained from Aladdin Chemical Reagent Co. Ltd. (Shanghai, China). Sodium hydroxide was purchased from Tianjin ZhiYuan Chemical Reagen Co. Ltd. PDGF-BB, bovine serum albumin (BSA), human serum, and mouse immunoglobulin G (IgG) were obtained from Solarbio Bioengineering Ltd. Company (Beijing, China). Aptamer was obtained from SBS Genetech Co., Ltd. (Beijing, China), and the sequence was: 5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3′. Thrombin from human plasma was obtained from Sigma-Aldrich Co. Ltd. The whole reagents were of analytical grade, and all solutions were prepared with Milli-Q ultrapure water (≥18.2 MΩ·cm).

The buffer was 0.1 M phosphate-buffered saline (PBS, pH 7.4). The aptamer stock solution (100 μm) was prepared in above PBS and stored at −4 °C. PDGF-BB and other protein stock solutions (1 mg mL^{-1}) were prepared by dissolving the proteins in PBS buffer (100 mM, pH 7.4) and further being diluted with the reaction buffer to obtain the desired concentration. The actual sample was obtained from the diluted human serum containing different PDGF-BB concentrations. The electrochemical behaviors of the modified electrodes were evaluated through cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) in 5 mM $K_3[Fe(CN)_6]$ of PBS solution containing 1 M KCl and 0.14 M NaCl.

2.2. Preparation of AgNCs@Apt, 3D-rGO@AgNCs@Apt, and GQD@AgNCs@Apt composites

The preparation procedure of three dimensional reduced graphene oxide (3D-rGO) was same as our previous work (Yang et al., 2015). Furthermore, graphene quantum dots (GQD) was obtained from 3D-rGO via oxidation with a mixture of concentrated HNO_3 and H_2SO_4 under stirring for 24 h and ultrasonication for 24 h at room temperature. Finally, the solution was dialyzed thrice with a dialysis bag (molecular weight: 8000 Da). Typically, 100 μL of 0.1 mg mL^{-1} $AgNO_3$ solution was added to 500 μL of aptamer (1 μm) in PBS with vigorous stirring at room temperature for 5 min. The mixture was kept in the dark at 0 ℃ for 3 h. Subsequently, about 100 μL of 2.3 mg mL^{-1} $NaBH_4$ was dropwise added to the above solution until the solution turned from colorless to brown, which indicated the formation of various amounts of clusters. As a result, the AgNCs@Apt composite was obtained. As for the preparation of the 3D-rGO@AgNCs@Apt and GQD@AgNCs@Apt composites, 0.01 mg of 3D-rGO and 1 mL GQD were added into the mixture of $AgNO_3$ and aptamer solution for 3 h, separately, according to the same procedure with the preparation of AgNCs@Apt composite.

2.3. Fabrication of electrochemical biosensors based on AgNCs@Apt, 3D-rGO@AgNCs@Apt, and GQD@AgNCs@Apt composites

Prior to modification, the surface of gold electrode was washed with piranha solution (H_2SO_4:H_2O_2=7:3) and rinsed in ethanol and water under ultrasonication for 5 min. The electrochemical biosensor was prepared through dropping 10 μL of pre-prepared AgNCs@Apt, 3D-rGO@AgNCs@Apt, or GQD@AgNCs@Apt onto the surface of the gold electrode. Briefly, the modified gold electrode was immersed in different concentrations of PDGF-BB for 2 h during electrochemical measurement.

2.4. Characterizations

X-ray photoelectron spectroscopy (XPS) analysis was obtained from an AXIS HIS 165 spectrometer (Kratos Analytical, Manchester, UK) with a monochromatized Al KR x-ray source (1486.71 eV photons). The surface morphology was determined using a high-resolution transmission electron microscope (TEM, JEOL JEM-2100) with a field emission gun of 200 kV. UV–vis spectra were obtained through a computer-controlled UV–vis spectrometer (TU-1201, Beijing Instrument Company).